AD 296 278

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TRANSLATION NO.

712

JANUARY 1963

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

FD 2-3742
JPRS: R-2950-D
January 1963

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U. S. JOINT PUBLICATIONS RESEARCH SERVICE
BUILDING T-30
OHIO DRIVE & INDEPENDENCE AVENUE, S. W.
WASHINGTON 25, D. C.

ON THE PRODUCTION OF CRYSTALLOMORPH CONCRETIONS OF A PROTEIDIC NATURE BY A STRAIN OF PASTEURELIA PESTIS

by

Georges Girard*

In a preceding article (G. Girard, Comptes Rendus de l'Academie des Sciences, Vol. 235, 1952, page 1441), we reported how the unexpected discovery of whitish concretions interspersed in cultures on nutrient gelatin from the EV strain of Pasteurella pestis (it is a question of the Girard and Robic EV strain, used as a plague vaccine, alive in Madagascar since 1934 as well as in other plague endemy territories) constituted, for twenty years, only a mere bacteriological curiosity. We refer to that article in so far as concerns equally unexpected conditions in which the phenomenon was repeated and how we were able to reproduce it, which allowed us to pursue its study.

In truth, the production of the concretions on the culture medium , that, we recall, is composed of macerated beef to which 20 g. of commercial peptone (Uclaf brand) and 5 g. of NaCl per liter were added, gelatinized at 20 per 1,000, was subject to variations that were capricious to say the least, and we succeeded, only after multiple observations, in determining the most favorable conditions for obtaining them.

First of all, the inoculations must be made on dry mediums, without a trace of condensation water. Then it is well to start with cultures that already have concretions formed from agglomerations of needles with a crystalline appearance containing plague bacilli that have remained viable, even after several months. A concretion is transplanted into peptonized water after it has been crushed and diluted in a tube of peptonized water, and this young (48-hour) culture will be used to inoculate tubes and Roux boxes that will be incubated at 24-28°C. for four to five days. Then they will be left uncovered in a cabinet at laboratory temperature. Fifteen to twenty days later for the tubes, three or four weeks for the boxes, the concretions form on the upper part of the medium, which is the driest, and then spread over the entire surface or only over part of it. Although a fortunate accident favored us with the creation of these formations by three clones derived from single cells (Girard, op. cit.), we ascertained, subsequently, that in certain tubes some colonies that stood out over the entire culture by their appearance were lacking those infiltrations or those whitish efflorescences that mark the beginning of the phenomenon we are describing. These colonies, transplanted under the same conditions as

*Presented at the 28 February 1959 meeting of the Biology Society [Societe de Biologie] and published in the Comptes Rendus de la Societe de Biologie No. 158, 1959, pages 277-279.

the others, produce no crystals. This heterogeneity, normal, moreover, in a microbian population, and especially well-known in so far as Pasteurella pestis is concerned from other points of view, can explain why there are, among the subcultures of the EV strain returned to us for checking by several laboratories, some that do not yield crystals or produce only a few, and that by exception.

These formations are evidently associated with the development of the culture of the EV strain, but they are exteriorized only on a par with the colonies themselves, as is evidenced by the tubes that contain only well isolated colonies, or even one only. Their absence in the lysis zone created by touching with a culture from a lysogenic strain, whereas they appear on the surface of the culture not touched with the lysis, also proves it. As J. Robic had seen as early as 1934, they are never formed in cultures whose tubes have been sealed after a few days, and if some tubes are sealed when they begin to appear on top, their development is completely arrested at that stage. Nevertheless, although desiccation together with aeration are the determining factors of the phenomenon as it is seen on our gelatin medium, the essential substance of the concretions is also formed in a liquid medium. We left a culture that had developed previously at laboratory temperature for six weeks, under incubation at 37°C. for several months. After evaporation, the sediment of the balloon was rich in crystals of the same nature as the ones produced in cultures on the gelatin medium. Nothing similar was observed in the culture of another strain of plague subjected, as a control, to identical conditions.

It is quite difficult to collect these concretions, for some of the gelatin, within which they are at times deeply anchored. always comes off with them. Washing the surface necessarily carries off the microbian culture incorporated in these formations or located in their interval. When we noticed that the crystals that are their dominant element were soluble in distilled water, it was easy for us to separate them from the plague bacilli by filtration over a candle. The addition of a saturated NaCl solution to the filtrate reprecipitates them with the appearance of bundles of fine needles. Our colleagues, G. Milhaud and J. P. Aubert, performed the first analyses on this material, the results of which they communicated to us and are reproduced here:

"The spectrum of the crystallized product in the ultraviolet band gives an absorption ratio 280 mμ/260 mμ of 1.8. On the other hand, after hydrolysis in hydrochloric acid for fourteen hours and chromatography on paper, the following amino acids have been identified up to now: aspartic and glutamic, alanine, leucine, isoleucine, valine, and lysine acids. These two analyses allow one to infer the proteidic nature of the crystallized product."

On his part, our colleague, P. Manigault (we express our sincere gratitude to Messrs. Manigault, Milhaud and Aubert for their friendly and valuable assistance), reports in a special article the findings that are derived from his microscopic study of the crystals, of which he has obtained some very fine photomicrographs.

The significance of the phenomenon escapes us completely, and we

refrain from formulating the slightest hypothesis with regard to it. It has in its favor its originality, for it is unique in microbiology in the form in which it appears. It is not connected with a degenerescence of the EV strain, since it was observed twenty-five years ago, at a time when its degree of virulence was rather attenuated for the preparation of a vaccine virus inoculated in man on a vast scale without any accident, when it had its maximum protective value. The phenomenon was never with other strains lacking in all virulence and toxicity, and, by virtue of this fact, non-immunizing. We do believe, however, that we can put forward the theory that there is a close correlation between the chemical constitution of the EV strain and the crystalline formations derived from it. We hope that new research of a chemical and immunological nature on a material that we are preparing to collect now in an appreciable amount will throw light on the problem that we have raised.

Conclusion:

A curious phenomenon, until now unique in microbiology, a strain of Pasteurella pestis (Girard and Robic EV strain), the only one in this microbian species, produces, by an unknown process, on a certain gelatin medium, crystalline concretions that it is possible, under the conditions reported by the author, to obtain regularly, whereas for a long time they were observed only intermittently. The analysis of these crystals shows that they are proteidic in nature.

The significance of the development is unknown and will be clarified only with new research on the purified material, in order to complete its chemical study and to procede to conduct research of an immunological nature.

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SUMMARY

For some twenty years the appearance of whitish concretions interspersed in cultures made on nutrient gelatin from the Girard Robic EV strain of Pasteurella pestis was merely a bacteriological curiosity. The author succeeded in determining the most favorable conditions for producing the concretions for the purpose of study. Desiccation together with aeration are the determining factors in producing the phenomenon on a gelatin medium. An analysis of the crystallized product reveals the identifiable presence of seven amino acids. This fact, together with the results of a spectral analysis, proves the proteidic nature of the concretions. The significance of this phenomenon is still unknown, but further research, now being conducted by the author, will probably throw more light on the problem.